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Review

Involvement of transient receptor potential proteins in cardiac hypertrophy

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Abstract

Cardiac hypertrophy is an adaptive process that occurs in response to increased physical stress on the heart. Hypertrophy, which may be induced by hypertension among other factors, is characterized by an increase in left ventricular mass and an associated increase in force production capacity. However, as sustained cardiac hypertrophy may lead to heart failure and sudden death, an understanding of the molecular processes involved in both the onset and consequences of hypertrophy is of significant importance. Calcium is a key player in the process underlying the development of cardiac hypertrophy. Recently, several Transient Receptor Potential proteins (TRPs), including calcium-permeable and calcium-regulated ion channels, have been shown to be related to various aspects of cardiac hypertrophy. TRPs are implicated in the development of cardiac hypertrophy (TRPC1, TRPC3, TRPC6), the electrophysiological perturbations associated with hypertrophy (TRPM4) and the progression to heart failure (TRPC7). This review describes the major characteristics of cardiac hypertrophy and focuses on the roles of TRPs in the physiological processes underlying hypertrophy.

Keywords: Cation channel; Cardiomyocyte; Heart; Hypertrophy; TRP; TRPC1; TRPC3; TRPM4

1. Cardiac hypertrophy and remodelling

1.1. Features of cardiac hypertrophy

Cardiac hypertrophy is characterized by an increase in left ventricular mass and associated changes in the shape of the left ventricle. It is an *in vivo* adaptive process that allows the organism to maintain or increase its cardiac output. Nevertheless, in humans, a sustained cardiac hypertrophy can lead to arrhythmias, heart failure and sudden death. Therefore, elucidation of the molecular processes involved in both the onset and consequences of hypertrophy is of great importance.

Hypertrophy is triggered by a wide array of processes in response to increased workload induced by hypertension, valvular dysfunction, myocardial infarction, genetic disease or endocrine disorders [1,2]. As illustrated in Fig. 1, these hypertrophy-causing conditions reactivate the foetal program in cardiomyocytes. Hypertrophy-related gene expression has been reported for (i) cytoskeletal constituents such as β-myosin heavy chain (β-MHC) and α-smooth muscle actin (α-sm actin); (ii) several types of proteins involved in ion transport such as the T-type calcium channel ($I_{Ca,T}$) and the hyperpolarization and cyclic nucleotide-activated channel (HCN); (iii) secreted factors such as atrial natriuretic factor (ANF) and angiotensin II; and (iv) transcriptional factors [1]. In contrast, the expression of other proteins like the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is repressed [3]. Modifications to the expression of these proteins can be manifested in terms of altered action potential shape, altered calcium transient properties and changes to the excitation–contraction coupling process. In addition, in cases of pronounced hypertrophy, the proliferation of fibroblasts can occur, thereby adding to the extent of cardiac dysfunction [4].

Concerning the implication of ion channels in cardiac hypertrophy, two points need to be considered. First, the phenomenon by which ion channels are involved in the induction of hypertrophy (mechano-sensitive signal); and second, modifications to the normal electrophysiological properties of cardiomyocytes, which consequently give rise to arrhythmias.

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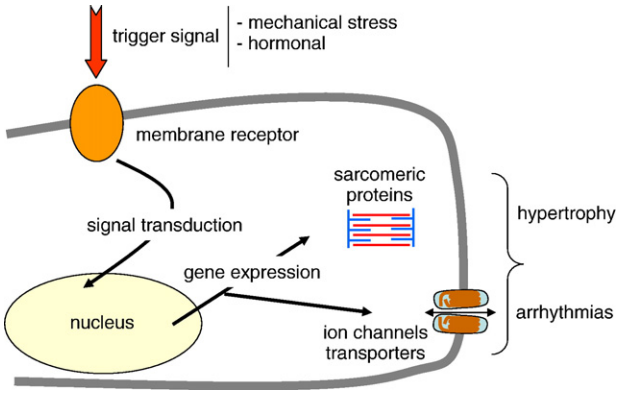

Fig. 1. Process of cardiac hypertrophy. Trigger signal for hypertrophy leads to the expression of sarcomeric proteins responsible for cardiomyocyte hypertrophy. Modifications in the expression of proteins involved in ion transport give rise to perturbations in the electrical activity of the cell.

1.2. Electrical modifications in cardiac hypertrophy

Among the modifications of gene expression induced by the hypertrophic process are included genes for several channel proteins involved in the electrophysiological properties of excitable cells. Alterations to these electrophysiological properties can be evident in the electrocardiogram (ECG), where a prolongation of the QT interval and also a modification of the cardiac axis in the direction of hypertrophied ventricle are common features associated with hypertrophy. Measurement of the QT interval provides a non-invasive indication of the action potential duration. Indeed, prolongation of the ventricular action potential, in association with a sustained depolarization, has been reported in numerous studies of hypertrophic remodelling [4–7]. While depolarization of the resting membrane potential is attributed to an alteration of the inward rectifier current I_{K1}, prolongation of action potential is mainly attributed to a reduction in the transient outward current (I_{to}) density [8]. In addition, the expression of other currents, such as the pacemaker funny current I_f [9,10] and the transient calcium current $I_{Ca,T}$ [11] is increased during hypertrophy.

Literature reports highlight the altered expression during hypertrophy of several proteins implicated in the Ca^{2+} transient; these include SERCA, the $I_{Ca,T}$ channel, the Na/Ca exchanger [3,11,12] and calcium-permeable non-selective cation channels (see following sections). The altered expression of these membrane proteins results in a prolongation of the calcium transient and an increase in the resting Ca^{2+} concentration. The modification of Ca^{2+}-dependant mechanisms appears to be the major source of arrhythmias in hypertrophied heart.

1.3. Calcium signalling pathways in cardiac hypertrophy

While mechanisms underlying the onset of hypertrophy remain largely unknown, Ca^{2+}, however, appears to be a major component of the phenomenon. An increase in calcium signalling, which is observed in the first stages of hypertrophy, is probably implicated in the induction of gene expression modifications (see [13] for review). Thus, driven by extrinsic factors, changes to the Ca^{2+} transient in terms of its frequency, amplitude or width are considered to be the primary signal for hypertrophy. Molecules that activate the phosphoinositide-specific phospholipase C (PLC) pathway may trigger modifications to the Ca^{2+} transient by the generation of 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) followed by the release of Ca^{2+} from the sarcoplasmic reticulum (SR). Protein expression induced by this Ca^{2+} release may operate through several Ca^{2+}-dependent mechanisms. Calcineurin, a Ca^{2+} and calmodulin-dependant protein phosphatase, was shown to be implicated in the integrating pathway (see [14,15] for review). Additional proteins transmit calcineurin-dependant signals to the nucleus with consequent changes in gene transcription. The most well-characterized substrate for calcineurin is the nuclear factor of activated T cells (NFAT). Calcineurin dephosphorylates serine residues of NFAT leading to its translocation from the cytoplasm to the nucleus where it engages a variety of transcription factors, leading to the activation of responsive genes. Several data suggest that the depletion of SR Ca^{2+} stores itself is not solely responsible for activating gene expression, but results in an influx of extracellular Ca^{2+} into the cytoplasm, causing a sustained elevation of the internal Ca^{2+} concentration. A link between Ca^{2+} stores depletion, activation of the capacitive Ca^{2+}-entry and modification of gene expression via the calcineurin/NFAT pathway was first demonstrated in T cells [16–18] and later in myocytes [19]. It was also observed in heart preparations where inhibition of store-operated Ca^{2+} currents prevents the calcineurin-dependant nuclear translocation of NFAT [20,21].

It should be noted that, in addition to the Ca^{2+}/calmodulin-dependant protein phosphatase calcineurin, the Ca^{2+}/*calmodulin*-dependant proteins kinases I, II and IV are also able to induce cardiac hypertrophy [22,23].

2. The Transient Receptor Potential (TRP) channel family

2.1. Molecular structure and regulation

From the above discussion, it appears that Ca^{2+} plays a crucial role in the development of cardiac hypertrophy and of its consequences. Thus the implication of Ca^{2+}-permeable channels in this process is of much interest.

The discovery of a novel family of cation channels, the "transient receptor potential" (TRP) protein family, has provided molecular support for a large variety of non-selective channels. The TRP cation channels, which were first characterized in tissue from the *Drosophila* eye, are classified for mammals into 6 subfamilies: TRPC (Canonical, 7 members), TRPV (Vanilloid, 6 members), TRPM (Melastatin, 8 members), TRPP (Polycystin, 3 members), TRPML (Mucolipin, 3 members) and TRPA (Ankyrin, 1 member) (see [24,25] for review). Most of the proteins of the main subfamilies (TRPC, TRPV, TRPM) are permeable to calcium and other cations ($P_{Ca}/P_{Na}=1–10$). However, some of these channels are strictly Ca^{2+}-selective (TRPV5, V6) and were presented as molecular candidates for the epithelial Ca^{2+} current I_{ECaC} [26]. Other members are highly Mg^{2+} selective (TRPM6 and TRPM7). In addition, those that are not Ca^{2+}-permeable (TRPM4 and TRPM5) were shown to be

regulated by Ca^{2+} [27,28]. Thus the majority of these channels could be implicated in calcium signalling. This point was recently extensively reviewed by Minke [26].

Concerning their molecular structure, all TRP channels are composed of subunits containing 6 transmembrane segments (TM1–6) that assemble as tetramers. The subunits have been shown to homo-associate but also, in some cases hetero-associate (see [24,25,29] for review). Thus great care should be taken when comparing channel properties observed in over-expressing systems and in native cells. Similarly to classical 6 TM channels, the channel pore, containing the selectivity filter and channel gate is formed by the assembly of the four loops linking TM5 to TM6. TRPs are considered as voltage-sensitive channels. However, the paucity of positively charged residues in TM4, the segment that confers voltage sensitivity to classical voltage-gated channels, suggests that the voltage sensitivity of TRPs uses a distinct mechanism. The intra-cytoplasmic C and N-terminal segments hold a variety of specific sites that differ from one TRP to the other and thus address specific regulatory properties. As such, a large variety of TRP activity modulators have been described in the literature and already extensively reviewed [24,25,29]. These modulators include intracellular or extracellular molecular components as well as biophysical modulators such as voltage or temperature. They work by activating G protein-coupled receptors or tyrosine kinase receptors; the production of DAG or IP_3 subsequently induces the liberation of Ca^{2+} from intracellular stores. Direct activation of TRPs by ligands has also been reported. These ligands consist of exogenous organic molecules such as capsaicin or menthol, endogenous lipids such as DAG or phosphoinositides, purine nucleotides such as ATP or ADP-ribose and inorganic ions such as Ca^{2+} or Mg^{2+}. TRPs are also regulated by a large number a kinases including PKA, PKC, PKG and the Ca^{2+}/calmodulin pathway.

2.2. Physiological implications of TRPs: calcium signalling and voltage modulation

Due to their large number and the diversity of their modulators, TRPs are implicated in a variety of physiological processes and serve, in particular, as signal integrators. In addition, these channels are considered as signal amplifiers. Indeed, they serve as molecular candidates for a variety of Ca^{2+} currents such as store-operated Ca^{2+}-entry channels (SOCs), receptor-operated Ca^{2+}-entry channels (ROCCs) and stretch-activated channels (SACs). In particular, TRPs may be implicated in the phenomenon called store-operated calcium entry (SOCE) involved in several cellular functions including responses that are dependant upon Ca^{2+} entry into the cell. Activation of plasma membrane receptors coupled to PLC leads to the production of IP_3, triggering intracellular calcium-store depletion. This depletion activates SOCE through plasma membrane proteins as SOCs. The pathway that links Ca^{2+} release from the SR to store-operated Ca^{2+} current activation is not known precisely. A number of mechanisms have been proposed, such as the diffusion of a soluble Ca^{2+} influx factor (CIF), the conformational coupling of activated IP_3-receptors to the channel mediating the Ca^{2+} entry, the fusion to the plasma membrane of cytoplasmic vesicles transporting Ca^{2+}-entry channels [30], and recently, involvement of the single spanning membrane protein STIM1 [31–33].

While still being debated, several lines of evidences support the idea that some TRPs may correspond to SOCs. Each of the TRP subfamilies likely hosts some members that can be activated by the depletion of intracellular calcium stores. This point will be detailed in the following sections concerning specific TRP channels implicated in cardiac hypertrophy.

TRP channels should also be regarded as membrane potential modulators. Because of their non-selective cation selectivity, opening of TRPs in a negative resting membrane potential will generally produce a depolarizing current. This property could be used in particular in excitable cells that possess a variety of voltage-sensitive channels that could thus be modulated by the membrane potential variation.

In the following sections, we focus attention on TRPs channels that have been implicated in cardiac hypertrophy, providing a brief description of their biophysical properties and reviewing their contribution to hypertrophy. This contribution should be regarded first as a pathway for extracellular Ca^{2+} entry into the cell, but also as a depolarizing component.

3. TRP channels in cardiac hypertrophy

In the heart, the presence of several TRPs – TRPC1, TRPC3–7, TRPV1, TRPV2, TRPV4, TRPM4, TRPM6, TRPM7 and TRPP2 – has been reported [34,35]. However, it is important to note that these channels were mostly detected by RT-PCR or by biochemical studies on the whole heart, meaning that the cellular expression profile and functional expression of the channels are thus yet to be determined. In fact, only six types of TRP currents have been recorded in the heart. These currents are associated with TRPC1, TRPC3 and TRPC6, three store-operated Ca^{2+}-channels expressed in mouse or rat ventricle implicated in calcineurin-dependant cardiomyopathies [36–39]; TRPM4, a Ca^{2+}-activated non-selective cation channel present in human atrial cardiomyocytes and ventricle from hypertrophied rat hearts [40,41]; and TRPM6 and TRPM7, which are responsible for a Mg^{2+}-inhibited cation current (I_{MIC}) in pig, guinea-pig and rat ventricular cardiomyocytes [42,43].

A large number of animal models of cardiac hypertrophy have been described in the literature, the majority of which have been developed in an effort to mimic the human condition (see [1,44] for review). These models include: (i) animals that have undergone a surgical procedure such as coronary ligation or aortic banding; (ii) animals that develop systemic hypertension after receiving a high-salt diet or injections of pharmacological agents; (iii) genetically selected animals such as spontaneously hypertensive rats (SHR) or the spontaneous hypertensive heart failure (SHHF) rat; (iv) development of adult rat cardiomyocytes in culture; and (v) transgenic animals that lack or over-express one of the proteins involved in the control of hypertrophy.

Several recent studies using these models showed the implication of TRPs in both the development of hypertrophy and its consequences including the triggering of arrhythmias.

Theses studies are reported in the following section for each TRP involved and summarized in Table 1.

3.1. TRPC1

One candidate for the SOCE pathway is TRPC1, which was the first member of the TRPC family to be cloned. This membrane protein is a 16 pS non-selective cation channel with similar permeabilities for Na^+ and Ca^{2+} and was shown to be activated by calcium store depletion when expressed in CHO or Sf9 cells [45]. Later, the implication of TRPC1 in SOCE and subsequent NFAT activation was demonstrated in B lymphocytes [46]. In two recent studies, Ohba et al. [38,39] similarly observed that overexpression of TRPC1 in HEK 293T cells increased SOCE, leading to a rise in NFAT promoter activity. Subsequently, using primary cultures of neonate rat cardiomyocytes, these authors observed an increase in the expression of TRPC1 and SOCE associated with cell hypertrophy induced by endothelin 1 treatment. This augmentation of TRPC1 expression associated with cardiac hypertrophy was also observed in abdominal aortic-banded rats that developed cardiac hypertrophy [39]. In contrast, silencing of the TRPC1 gene via small interfering RNA (siRNA) attenuates SOCE and prevents cardiac hypertrophy [39]. These results highlight the role of TRPC1 as an important regulator of cardiac hypertrophy through its SOC properties.

3.2. TRPC3

Another candidate to support SOCE is TRPC3. This protein is a non-selective cation channel with a single channel conductance around 25 pS [30]. It is a poor discriminator of Na^+ over Ca^{2+} ($P_{Ca}/P_{Na}=1.6$). In neurons, TRPC3 appears to be an integral component of a signal transduction cascade involving PLCγ, the generation of IP_3 and an increase in $[Ca^{2+}]_i$ [47]. It belongs to the DAG-sensitive TRPC3/6/7 subfamily. While its classification as a SOC channel is still being debated (see [30] for review), activation of NFAT was shown to be increased in myocytes transfected with TRPC3 expression plasmid [19].

The contribution of the TRPC3 channel to capacitive Ca^{2+} entry has recently been highlighted in two cardiac models. In one study, Bush et al. [48,49] used microarray analysis to examine the pattern of expression of a variety of genes from cultured cardiomyocytes exposed to the α-adrenergic receptor agonist phenylephrine, which is considered capable of inducing cellular hypertrophy. Among the genes most potently modified, TRPC3 is upregulated. Using several animal models of hypertrophy, including cultured neonatal rat ventricular myocytes, SHHF rats and isoproterenol-induced heart hypertrophic rats, Bush et al. [48] investigated the contribution of the TRPC3 channel to the development of hypertrophy. They used an adenoviral strategy to induce the over-expression of TRPC3 in cultured cardiomyocytes, and observed a weak sarcomere assembly that was increased in the presence of oleoyl-2-acetyl-*sn*-glycerol (OAG), a molecule known to activate several TRP channels including TRPC3 [50]. In addition, TRPC3 over-expression produced an increase in cell volume, as well as an upregulation of ANF and α-sm actin mRNA transcripts, two markers of hypertrophy. The use of the SOC inhibitor 2-aminoethoxydiphenylborane (2-APB) reduced the α-adrenergic receptor agonist-stimulated ANF secretion and cell volume increase. Furthermore, a link between TRPC3 and calcineurin was demonstrated by immunoblotting studies showing that TRPC3 stimulates NFAT. Together, the results demonstrate that TRPC3 expression is increased in hypertrophy, and that it is implicated in the development of hypertrophy via calcineurin/NFAT signalling.

The implication of TRPC3 in hypertrophy was also elegantly demonstrated by another group using TRPC3 over-expressing transgenic mice [36]. Adult cardiac myocytes isolated from these mice showed abundant store-operated Ca^{2+}-entry that was inhibited by SKF96365, a known inhibitor of SOCs. In addition, transgenic mice showed an increase in NFAT activation *in vivo*, as well as cardiomyopathy and augmented hypertrophy after neuroendocrine agonist application or

Table 1
Incidence of TRP channels in cardiac hypertrophy

Model	Implication in hypertrophy	Reference
Endothelin 1 treatment of neonate rat cardiomyocytes in culture	Induced Hyp., increased SOCE Increased TRPC1 expression	[39]
Aortic abdominal banded rats	Induced Hyp., increased TRPC1 expression	[39]
Neonate rat ventricular cells exposed to Hyp. stimulation	Increased TRPC3 expression Implicated in capacitive Ca2+ entry	[48]
Isoproterenol-induced Hyp.	Induced Hyp. through calcineurin signalling	[48]
SHHF rats	Involving TRPC3 activation	[48]
Transgenic mice over-expressing TRPC3		[36]
Down-regulation of SERCA expression in cultured neonate rat myocytes	Decreased TRPC5 and TRPC4 expression	[53]
Rat neonatal cardiomyocytes in culture	Induced Hyp. by DAG stimulation of TRPC3,6	[37]
Transgenic mice over-expressing TRPC6	Increased NFAT-dependant expression of MHC	[52]
Transgenic mice harbouring an α-MHC-calcineurin transgene	Increased TRPC6 expression	[52]
Neonate rat cardiomyocytes transfected to over-express TRPC7	Increased apoptosis	[79]
Cultured adult rat cardiomyocytes that develop cell hypertrophy	Increased TRPM4 expression - implicated in DADs?	[66]
SHR	Increased TRPM4 expression	[41]

Models used to investigate the incidence of TRPs in cardiac hypertrophy. Hyp.=hypertrophy.

pressure overload stimulation. As proof of calcineurin involvement, the cardiomyopathic phenotype and the increased hypertrophy after pressure overload stimulation were blocked by targeted disruption of the calcineurin Aβ gene. In contrast, siRNA silencing of TRPC3 in cultures of rat neonate cardiomyocytes suppresses angiotensin II-induced NFAT translocation [37]. A schema summarizing the possible role of TRPC3 in cardiac hypertrophy is proposed in Fig. 2.

In association with TRPC3, it is also probable that the Na^+/Ca^{2+} exchanger participates in the increase of $[Ca^{2+}]_i$. Indeed, it was reported that a local coupling existed between the two proteins when expressed in HEK293 cells [51]. Ca^{2+} entry into TRPC3-expressing cells involves the reversed mode of the Na^+/Ca^{2+} exchanger. In addition, co-immunoprecipitation experiments provided evidence of the association of these two proteins.

3.3. TRPC6

In addition to TRPC1 and TRPC3, TRPC6 was also recently revealed to be involved in calcineurin-NFAT signalling both upstream and downstream of NFAT. Kuwahara et al. [52], followed the cardiac expression of TRPCs using transgenic mice harbouring an α-MHC-calcineurin transgene, which results in cardiac hypertrophy. They detected TRPC1,3,4,6 transcripts and among these observed that TRPC6 expression was upregulated in the hypertrophied hearts. Consistent with these findings, the promoter of the TRPC6 gene contains 2 NFAT-binding sites. On the other hand, cardiac-overexpression of TRPC6 in transgenic mice resulted in an increase in NFAT-dependant expression of β-MHC, considered as a marker of pathologic hypertrophy [52]. At the opposite, siRNA knockdown of TRPC6 reduced hypertrophic signalling induced by phenylephrine and endothelin-1 [52]. These data are in accordance with the implication of the channel in activation of the calcineurin-NFAT pathway in response to G protein-coupled receptor signalling. The pathway that links G protein to channel activation was investigated in rat neonatal cardiomyocytes. In this model, the PLC-mediated production of DAG was found to be essential for Angiotensine II-induced NFAT activation [37]. TRPC3, TRPC6 and TRPC7 belong to the DAG-activated TRP channels. The activity of TRPC3 and TRPC6 seems to be correlated with Angiotensine II-induced NFAT activation and hypertrophic process but not TRPC7. The effects of these channels may include both an increase in calcium entry through the channel as well as membrane depolarization, which activates voltage-gated calcium permeable channels such as the L-type Ca^{2+} channel [37].

3.4. TRPC4 and TRPC5

A crucial role in modifying $[Ca^{2+}]_i$ in cardiac hypertrophy has been demonstrated, until now, only for TRPC1, TRPC3 and TRPC6. However, experimental data suggest the possible contribution of other TRP members. The reduction of SERCA expression during hypertrophy (see Section 1.1) could induce an increase in TRPC4 and TRPC5 expression. Indeed, down-regulation of cardiac SERCA2 in cardiomyocytes in culture using interfering RNA is followed by increased transcription of the Na^+/K^+ exchanger, TRPC4, TRPC5 and NFAT [53]. Unfortunately, TRPC3 expression was not investigated in that study. However, the study's findings suggest a link between the regulation of Ca^{2+} reuptake and expression of proteins involved in $[Ca^{2+}]_i$ regulation. One could therefore postulate that TRPC4 and TRPC5 are implicated in the process. Interestingly, it was observed that TRPC5 mRNA and protein expression were significantly elevated in failing human heart relative to non-failing controls [48]. In contrast, TRPC3 was detectable in

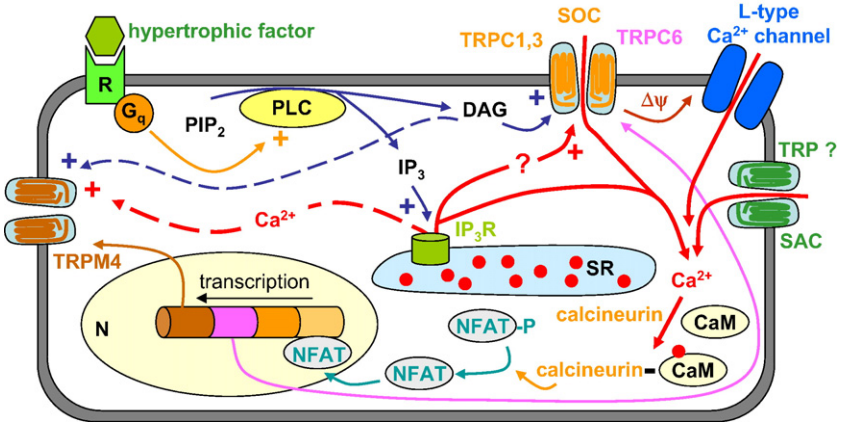

Fig. 2. Implication of TRP channels in the proposed mechanism of cardiac hypertrophy. After binding of a hypertrophic factor to a G_q protein-coupled receptor (R), the activated phospholipase C (PLC) cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol-1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). Ca^{2+}-depletion of the sarcoplasmic reticulum (SR), triggered by the binding of IP_3 to its receptor (IP_3R), activates the TRPC3,6 store-operated channels (SOC) by an unknown mechanism. Direct activation by DAG is also involved. Activation of TRPC1,3,6 produces an inward depolarizing current, thus modifying the membrane potential (Δψ) in favour of the activation of L-type Ca^{2+}-currents. Capacitive Ca^{2+}-entry through TRPC1,3,6, in addition to the Ca^{2+} provided by SR depletion and L-type Ca^{2+}-currents, activates calmodulin (CaM) which couples with calcineurin to dephosphorylate nuclear factor of activated T cells (NFAT). Ca^{2+}-permeable, stretch-activated channels (SACs) may also contribute to the rise in intracellular calcium. Once activated, NFAT is able to enter the nuclear compartment (N) to induce gene expression. One of these genes is for the TRPC6 protein. Another may be for the TRPM4 channel, which carries a depolarizing current activated by the elevated levels of Ca^{2+} and DAG in the sarcoplasm, thus inducing arrhythmias.

neither non-failing nor failing human heart. Thus it could be suggested that, due to species specificity, TRPC5 might be expressed during human heart remodelling as opposed to TRPC3 in rat or mouse.

In addition to their role in the induction of cardiac hypertrophy, TRPs could also provide new influx pathways for both Na^+ and Ca^{2+} during the action potential and thus modify its shape. The relation between the reduction of SERCA, a hallmark of cardiac hypertrophy, and the rise in the expression of TRP proteins (TRPC4–5) is also a source of potent electrophysiological perturbations [53].

3.5. TRPM4

A major cellular perturbation during cardiac hypertrophy is the increase in $[Ca^{2+}]_i$. This induces an activation of Ca^{2+}-dependant mechanisms, including the activation of Ca^{2+}-sensitive channels. Among these, we reported a functional overexpression of the TRPM4 protein in rat ventricular cardiomyocyte during hypertrophy [41].

TRPM4 is a calcium-activated non-selective cation channel. When expressed in HEK293 cells, it displays a linear current/voltage relationship with a conductance of 25 pS [27]. It is activated by membrane depolarization [54] and by an increase in $[Ca^{2+}]_i$ for which the concentration for half maximal activation ranges between 0.4 [27] and 9.8 μmol/L [55]. This Ca^{2+} sensitivity is modulated by PKC-dependant phosphorylation [56]. The channel is equally permeable to Na^+ and K^+, but in contrast to all other TRPs except TRPM5, it is not permeable to Ca^{2+}. It is blocked by intracellular adenine nucleotides including ATP. These properties are in accordance with its molecular structure, which displays a range of functional sites in its intracellular loop domains, such as six PKC phosphorylation sites, four ATP binding sites and five calmodulin binding sites [56]. TRPM4 mRNA is expressed in a variety of mammalian cells with a heightened expression in the heart and kidney [27,54]. Taken together, the TRPM4 fingerprint matches very closely the properties of several Ca^{2+}-activated non-selective cation currents described in a large variety of tissues [57]. In the heart, it was detected in human atrial cells [40] and more recently in mouse sinoatrial node cells (see [58,59] for review). In the ventricular cells, its expression is amplified during cardiomyocyte hypertrophy.

Our first indication that TRPM4 is over-expressed during cardiac hypertrophy came from work done on adult rat ventricular cardiomyocytes in culture. This model is a suitable *in vitro* model for detailed analysis of hypertrophy at the cellular level. Indeed, during the culture, the foetal program of gene expression is reactivated, mimicking events associated with hypertrophy in vivo [60–65]. While absent from freshly isolated cells, TRPM4 was detectable in more than half of the excised patches from dedifferentiated cells after 1 week of culture [66]. As indicated in Fig. 3, an increase in cell capacitance as the time in culture progresses provides an indication of hypertrophy. At the same time, the resting membrane potential becomes less negative and spontaneous beating is observed after 1 week in culture [61,62,64,67,68]. Depolarization of the normal resting membrane potential is consistent with the modifications made

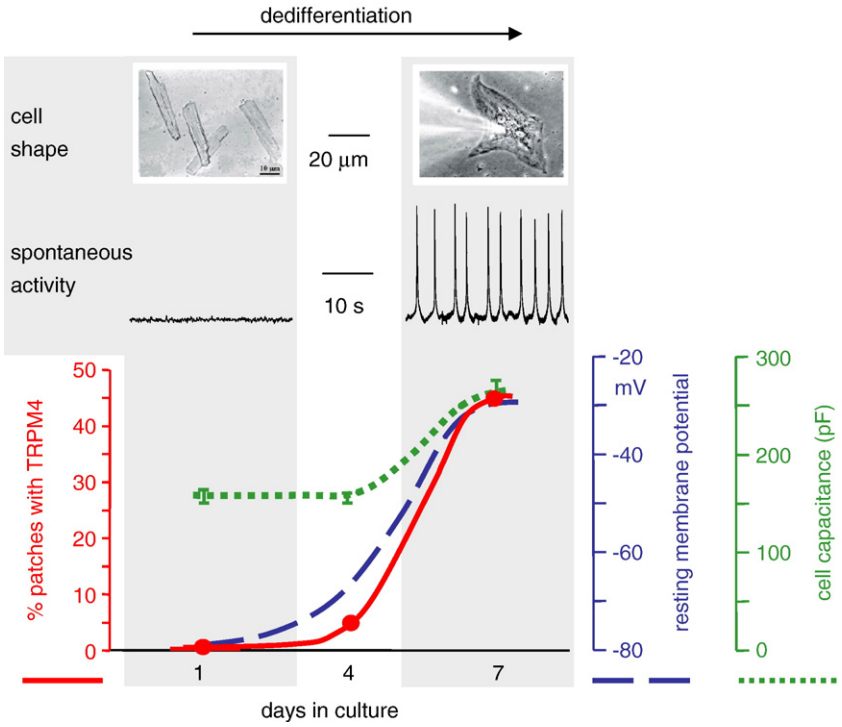

Fig. 3. TRPM4 expression induced by hypertrophy of cardiomyocytes in culture. Adult rat ventricular cardiomyocytes undergo a process of hypertrophy with the first week in culture as shown by an increase in cell capacitance and strong modification of cell shape [61]. Spontaneous electrical activity develops after cell remodelling and, by that time, the resting membrane potential becomes less negative [68]. These phenomena are correlated with an increase in TRPM4 functional expression [66,69].

by pacemaker currents (I_f and $I_{Ca,T}$) that are re-expressed during cell dedifferentiation, together with a reduction in the background potassium current (I_{K1}). Nevertheless, it is feasible that the TRPM4 current also participates in the mechanism of membrane potential depolarization, which in turn would explain the triggering of spontaneous beating. TRPM4 could also be implicated in the prolongation of action potential duration reported to be present in the hypertrophied heart. Interestingly, for the myocyte culture model, we also observed that the Ca^{2+}-activated non-selective cation current is activated by DAG analogues. This is achieved not only by PKC-dependent activation, but also by a PKC-independent pathway that is probably a direct interaction of the analogues, as reported for other TRP channels [69]. These regulations should be physiologically significant given that both DAG content and PKC activity increase during hypertrophy [70,71].

Further investigations have confirmed TRPM4 expression during cardiac hypertrophy by using freshly isolated ventricular cardiomyocytes from spontaneously hypertensive rats (SHR), a well-established genetic model of hypertension and cardiac hypertrophy when compared to its normotensive equivalent, the Wistar–Kyoto (WKY) rat. We functionally detected the current and also studied the presence of TRPM4 mRNA in ventricular tissue from both SHR and WKY rats [41]. The level of TRPM4 mRNA is much higher in SHR than in WKY rats. In parallel we observed an increase in functional detection of the TRPM4 current (Fig. 4). Channel detection was proportional to the heart-to-body weight ratio, showing that TRPM4 detection is not due to genetic selection of the rat population but rather related to the development of hypertrophy. TRPM4 is thus expressed during ventricular remodelling in SHR and could participate in some features of cardiac arrhythmias associated with hypertrophy.

Minimal data are available concerning Ca^{2+}-activated non-selective cation currents at the macroscopic level, probably due

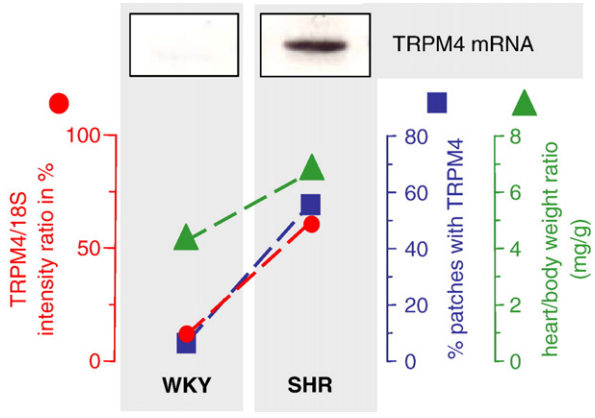

Fig. 4. TRPM4 expression in ventricular cardiomyocytes from SHRs. While TRPM4 mRNA is weakly detected in ventricular myocytes from normotensive WKY rats, it is highly detectable in tissue from hypertensive SHR animals that develop cardiac hypertrophy as shown by the increase in heart/body weight ratio. This expression is functional as TRPM4 current is recordable in SHR but not in WKY cardiomyocytes. It is postulated that cardiac hypertrophy induces TRPM4 expression which in turn participates in the triggering of arrhythmias [41].

to the difficulty involved to clearly isolate these currents from other cationic components. However, the implication of such currents in delayed after-depolarizations (DADs) has been reported [72]. DADs are depolarizations that follow the action potential. When sufficiently large and frequent, DADs can induce additional action potentials and thus arrhythmias. DADs occur because of the development of the calcium-dependant transient inward current (I_{ti}) induced by calcium waves, as have been described to appear in SHR myocytes during the development of hypertrophy [73]. I_{ti} has been extensively studied in cardiac cells and shown to have three components: the Na^+/Ca^{2+} exchanger, a $[Ca^{2+}]$-activated chloride current and a $[Ca^{2+}]$-activated non-selective cation current [59]. In a rat model of cardiac hypertrophy induced by injections of isoproterenol, it has been shown that the incidence of DADs increases with development of hypertrophy and that the Ca^{2+}-activated non-selective cationic component is responsible for an estimated 13% of the overall amplitude of DADs [74]. TRPM4 appears as a good candidate for this last component. On that note, it is important to point that the macroscopic non-selective cationic component of I_{ti} was detected in human atrial myocytes but not in human ventricular cells [75]. This finding is in accordance with our results. Indeed, in human atria, we detected TRPM4 single channel currents but no current or only weakly expressed current in normotensive rat ventricles [40,66]. Also, in rat, TRPM4 mRNA expression was higher in atria than in ventricles [41]. Thus, we hypothesize that TRPM4 may participate in I_{ti} in atrial but not ventricular normotensive rat cardiomyocytes. On the other hand, TRPM4 may contribute to this current component in hypertrophied ventricular cells and, thus, participate in the increased appearance of DADs during cardiac hypertrophy.

TRPM4 could also be implicated in the pro-arrhythmic early after-depolarizations (EADs) that occur during the action potential. These are associated with secondary Ca^{2+} release from the SR and occur at depolarized potentials when the TRPM4 current is supposed to be fully activated [76]. In that sense, it has been reported that ventricular hypertrophy induces EADs in rabbit [77]. It is important to mention that depolarization induced by TRPM4 activation may, as was specified for TRPC3, activate voltage-sensitive Ca^{2+}-entry pathways and thus contribute to the modifications of calcium signalling seen during hypertrophy.

3.6. TRP channels and the end-point of heart failure

With the passage of time, compensated hypertrophy progresses to cardiac failure. As apoptotic cells are more abundant in the failing than in the non-failing heart, it has been suggested that apoptosis might be a mechanism involved in the development of cardiac failure after hypertrophy [78]. It has been recently shown that angiotensin II stimulation of cultured neonatal rat cardiomyocytes transfected with the Ca^{2+}-permeable TRPC7 channel exhibited a significant increase in apoptosis due to activation of the angiotensin II type 1 receptor [79]. Considering that the level of angiotensin II is increased during hypertrophy, at least in SHR [80], and that TRPC7 is abundantly expressed in heart [35], one

can postulate that the angiotensin II/TRPC7 link might be a key mechanism in the process leading to heart failure following cardiac hypertrophy.

It was also reported that TRPC6 mRNA expression was augmented in hearts of human patients with dilated cardiomyopathy compared with nonfailing hearts [52].

4. Conclusion

In conclusion, while mechanisms involved in cardiac hypertrophy have been under investigation for a long time, only a relatively small number of reports link the TRP family to cardiac hypertrophy. This is due, however, to the fact that the TRP proteins have only been recently cloned. The reports to the present time should be regarded as the initial steps in a field that is likely to expand significantly in the future. On this note, most of the reports that associate TRPs to cardiac hypertrophy were published at the end of 2006 [36–39,41,48,52,79]. The development of specific pharmacological tools and of transgenic animal models for each TRP would help to elucidate their function and confirm or identify those TRPs likely to serve as therapeutic targets in the treatment of cardiac hypertrophy and heart failure.

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